

SHORT COMMUNICATION

First report of prunus necrotic ringspot virus infecting rose (*Rosa* spp.) in Lebanon

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Summary Prunus necrotic ringspot virus (PNRSV) was detected in several rose plants showing symptoms of rose mosaic disease (RMD) in Beqaa valley, Lebanon. PNRSV was found in 29 plants by molecular and serological analyses, while other viruses associated with RMD were absent. Although PNRSV is known to have a wide host range, the present paper reports the first occurrence of PNRSV on rose plants in Lebanon.

Additional keywords: PNRSV, RMD, RT-PCR, Sequencing

Rose (*Rosa* spp.), a woody perennial flowering plant, belongs to *Rosa* genus, in the family Rosaceae and is considered the most common type of flowering shrub. More than 200 species belong to the *Rosa* genus, of which more than half are polyploids (Fougère-Danezan *et al.*, 2015). Rose is the most popular ornamental plant with high economic and cultural importance as garden plant and raw material in the perfume industry in Lebanon as well as worldwide.

Rose mosaic disease (RMD) is generally caused by viruses belonging to two genera (i) *Ilarvirus* [prunus necrotic ringspot virus (PNRSV) and apple mosaic virus (ApMV)] and (ii) *Nepovirus* [arabis mosaic virus (ArMV) and strawberry latent ringspot virus (SLRSV)]. These viruses may occur in rose plants in single or mixed infections. In general, variable symptoms can be associated with RMD, depending primarily on rose cultivars, virus agents and environmental factors (Chen *et al.*, 2022). Symptoms on leaves usually asso-

ciated with RMD include ring spots and intense yellow spots, severe distortion and mottling, chlorotic line patterns, yellow vein banding and puckering (da Silva *et al.*, 2018). Low flower production and flower deformation sometimes accompanied by reduced size are also observed. Infected plants drop leaves easily in autumn and become more sensitive to cold (Cochran, 1972; 1982; Secor *et al.*, 1977; Thomas, 1984).

During spring 2021, RMD symptoms consisting of chlorotic zigzag pattern, squiggly discoloration with jagged edges on leaves and/or ringspots were observed on several red rose plants (*Rosa* spp.) in Douris, Zahle, Kab-Elias and Saghbine (Beqaa valley), Lebanon (Figure 1). Leaf samples from 29 symptomatic plants were collected and subjected to enzyme-linked immunosorbent assay (DAS-ELISA) tests, using commercial antibodies (LOEWE, Germany) against PNRSV, ApMV and ArMV, which are the three most common viruses associated with RMD (Yardimci and Çulal, 2009). Asymptomatic rose seedlings taken from a nursery located in Beqaa Valley were included as negative controls. All symptomatic plants reacted positively with the PNRSV antiserum, while ArMV and ApMV were not detected, thus excluding the occurrence of mixed infections with these viruses. The presence of PNRSV was confirmed by RT-PCR us-

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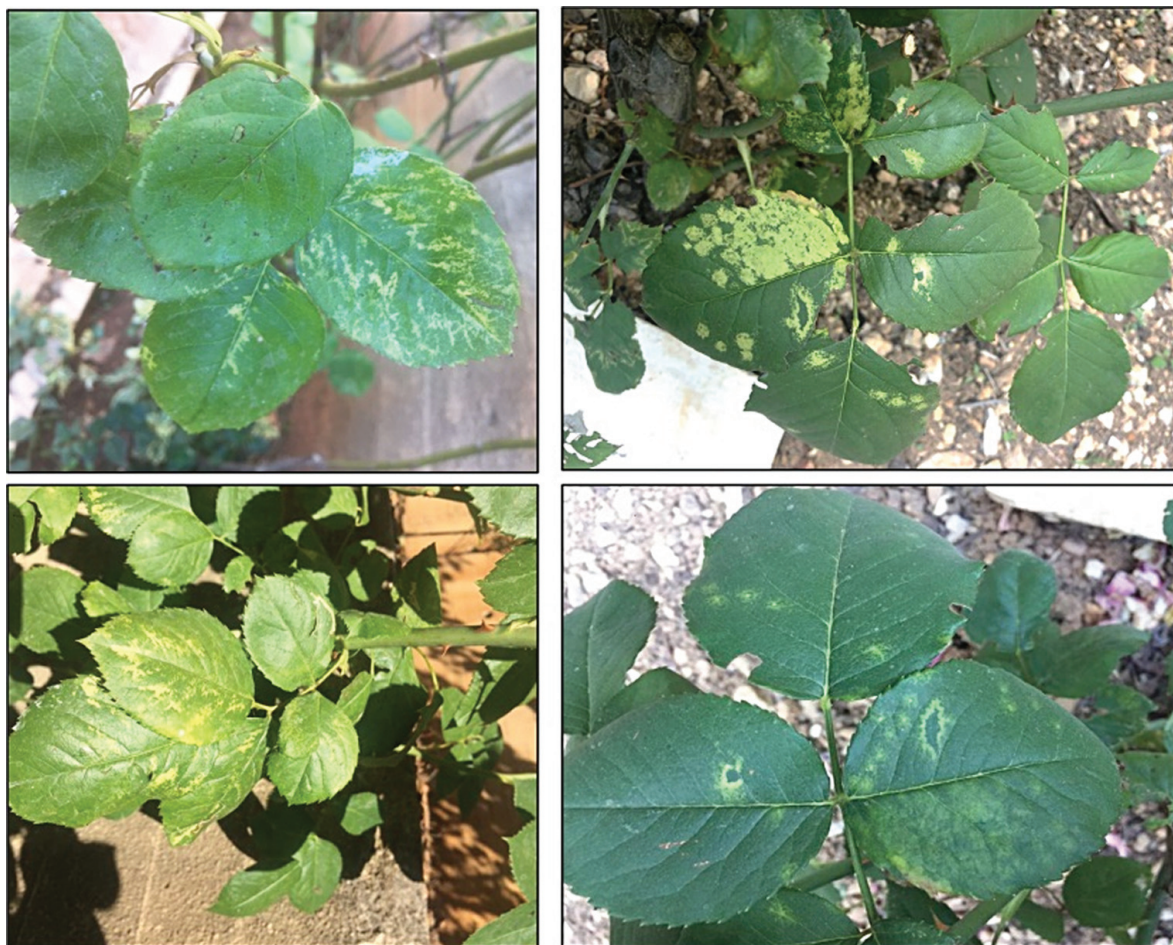


Figure 1. Symptoms of rose mosaic disease (RMD) caused by PNRSV on naturally infected rose plants in Lebanon.

ing primers: NRSCPF3/NRSCPR4 (Malinowski and Komorowska, 1998). Two amplicons (700 bp) were selected, purified by QIAquick PCR Purification Kit (Qiagen, Germany) and sequenced (Macrogen, Korea) in both directions. Bioinformatics tools used for sequence analyses and for local alignments at nucleotide level included NCBI BLAST tool (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>), BioEdit (Hall, 1999) and MEGA11 (Tamura *et al.*, 2021) software. Sequences from the two different isolates showed high similarity (99.8%) at nucleotide level.

The Lebanese isolate PNRSV-M2 was deposited in GenBank under accession number OM650244. Blast analysis revealed 99.2% nucleotide identity with isolates RM-2 (AY948440) and RM-5 (AY94841), two PNRSV isolates causing oak leaf pattern and mosaic symptoms on rose in India. Moreover, a total of 13 different isolates of PNRSV were ob-

tained from NCBI database in order to assess the phylogenetic relationships between the detected PNRSV isolate from Lebanon and other isolates distributed all over the world. Phylogenetic analysis clustered the Lebanese isolate in the same clade with the two isolates from India (Figure 2A). These results were further confirmed by a sequence dramatic tool (SDT) that can show pairwise identity scores using a color-coded pairwise identity matrix (Muhire *et al.*, 2014). Nucleotide identities between PNRSV-M2 and isolates from other countries are shown in Figure 2B. PNRSV has been isolated in many rose growing regions worldwide (Rakhshandehroo *et al.*, 2006). In Lebanon, PNRSV has already been reported on peach trees (Choueiri *et al.*, 2001) showing high level (61.2%) of infection.

To our knowledge, this is the first report of natural occurrence of PNRSV on rose

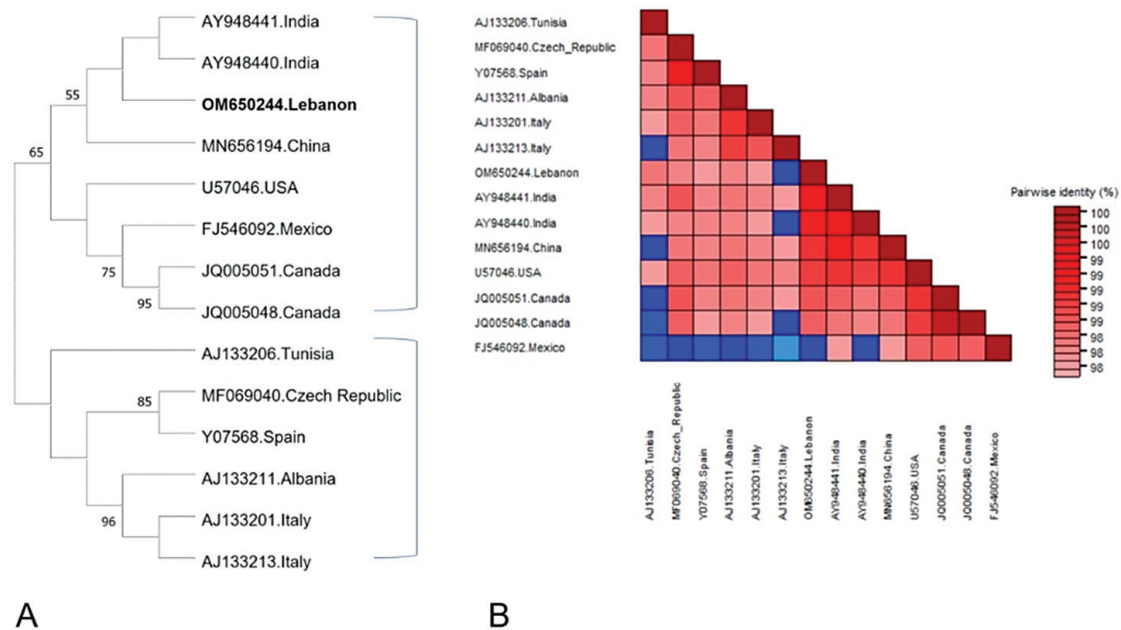


Figure 2. (A) Phylogenetic tree of prunus necrotic ringspot virus (PNRSV) isolates constructed by the Neighbor-Joining method using the 700 nucleotide sequences of the coat protein genes. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1,000 replicates) is shown next to the branches. Only node values above 50% are shown. Evolutionary analyses were conducted in MEGA11 (Tamura *et al.*, 2021), (B) Color-coded pairwise identity matrix generated using the Sequence Demarcation Tool (SDT) software by alignment of 700 bp fragment from CP gene of PNRSV isolate from Lebanon and another 13 isolates distributed worldwide. Each colored square represents a percentage identity score between two sequences. The colored scale key indicates the correspondence between the colors appeared in the matrix and pairwise identities between isolates.

plants in Lebanon. This finding rises important issues concerning rose production. In fact, PNRSV is difficult to control, because it is transmitted in several modes, mainly by grafting (Seitadzhieva *et al.*, 2022), by seeds and by some pollinator insects. This is additive to the absence of certified virus-free plants, which is one of the main problems in commercial rose production in Lebanon. Phytosanitary aspects need to be addressed in the next future to limit spread of PNRSV and further studies need to be undertaken to evaluate the impact of PNRSV on rose plants in Lebanon, as well as on other host plant species in the country.

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ΣΥΝΤΟΜΗ ΑΝΑΚΟΙΝΩΣΗ

Πρώτη αναφορά του ιού της νεκρωτικής δακτυλιωτής κηλίδωσης των πυρηνοκάρπων σε φυτά τριανταφυλλιάς (*Rosa* spp.) στον Λίβανο

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Περίληψη Ο ιός της νεκρωτικής δακτυλιωτής κηλίδωσης των πυρηνοκάρπων (*prunus necrotic ringspot virus*, PNRSV) ανιχνεύθηκε σε φυτά τριανταφυλλιάς που εμφάνιζαν συμπτώματα της ασθένειας του μωσαϊκού της τριανταφυλλιάς (RMD) στην κοιλάδα Beqaa, Λίβανος. Ο PNRSV βρέθηκε σε 29 φυτά με μοριακές και ορολογικές αναλύσεις, ενώ δεν ανιχνεύτηκαν άλλοι ιοί που σχετίζονται με το μωσαϊκό της τριανταφυλλιάς. Αν και είναι γνωστό ότι ο PNRSV έχει ευρύ φάσμα ξενιστών, η παρούσα εργασία αποτελεί την πρώτη αναφορά εμφάνισης του PNRSV σε φυτά τριανταφυλλιάς στον Λίβανο.

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